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A BACTERIOLOGICAL METHOD USEFUL FOR THE STUDY OF OTHER MICRO-ORGANISMS

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We have been using in some studies of bacteria for the past two years a method which appears to be equally valuable for the study of other micro-organisms. The bacteria are grown in a thin film of medium upon a microscopic slide and later stained. It was devised by Frost[1] in his studies on the bacteriology of milk in order to obtain a more rapid method of counting living bacteria in milk than the ordinary or standard plate method. It is invaluable not only to determine quickly the number of organisms in any richly seeded material, but also for a study of the morphology of the single cells or of the colonies. Very beautiful preparations may be obtained which show the relation of the cells to each other as well as the cytology of the individual cells. Recently I have applied this method slightly modified to the study of the yeast cell with very gratifying results.

Thaxter's potato hard agar, which consists of potato broth with 3 percent agar and 2 percent dextrose, is a favorable medium for the growth of yeasts. It is important that the medium be very clear. This is accomplished by the addition of egg albumen, followed by boiling and filtering according to the usual methods of procedure in the making of media. In this way it is possible to obtain a clear amber liquid which becomes only slightly less clear as it solidifies. This is introduced in approximately 5 cc. amounts in test tubes and sterilized.

When the plates are made, the slides are first thoroughly cleaned to free them from grease or other adhering material, then sterilized in the flame from a Bunsen burner and then placed on a warm stage. The temperature of the slides should be somewhat above the solidifying point of the medium used. The agar is liquefied and cooled to not lower than 45 °, then inoculated with about 1/10 cc. of a rather heavy suspension of yeast cells in water. These are evenly distributed in the medium by drawing up the fluid into the pipette several times. Approximately 1/20 cc. of the inoculated agar is placed on a slide. Another slide is placed on top of it, so that about three fourths of the surface of the slides are in contact. This spreads the medium quite evenly on the two slides. They are then drawn apart and at once placed in a moist chamber. The agar solidifies in a very few minutes. When only a few slides are prepared, I have found it very convenient to place them in Petri dishes which are lined with wet filter paper. In so small a container the atmosphere becomes saturated with moisture in a very few minutes and later the agar film shows no indication of drying. If very many slides are prepared it may be found more convenient to use some device like the specially prepared cabinet described by Frost.[2]

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[1] Frost, W. D. A Rapid Method of Counting Living Bacteria in Milk and Other Richly Seeded Materials. Jour. Amer. Med. Assoc. **46**: 889–890. 1916.

The slides in the moist chambers are incubated until the colonies have grown to the desired size. The cells multiply rapidly, resulting in colonies of various sizes. The films are then ready to be fixed and stained. In the process of fixing, washing with water, staining, dehydrating, etc., it will be necessary to exercise great care that the delicate film of agar does not float off the slide. For fixing the cells I have used picro-formol and Flemming's weak solution diluted one half. The slides are placed in a staining jar with the fixing fluid cover-

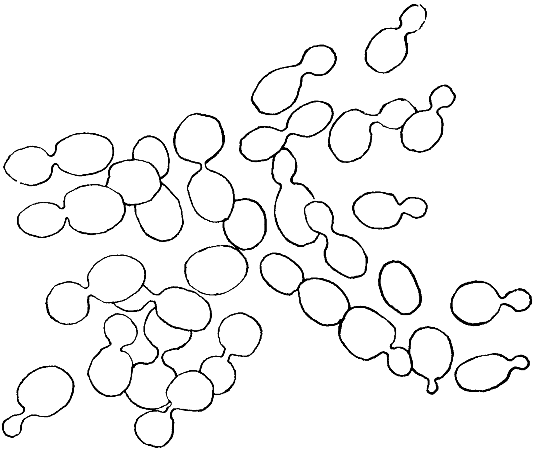

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FIG. 1. Yeast colony, grown, fixed and stained on microscopic slide.

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[2] L. c.

ing about half of the slide. This allows a part of the agar film, which is above the liquid, to dry firmly on the slide and so helps to prevent loss of the film. If the entire edge of the film becomes loose from the slide, the film is certain to be lost. I have introduced and removed all liquids rather slowly and very carefully with a pipette in order to prevent a rapid movement of the liquid from tearing the film. Sometimes a portion of the film will be torn off, but more often the film remains attached to the slide throughout.

After fixing, the films are washed and then stained. I have used Heidenhain's iron hematoxylin with excellent results. After staining,

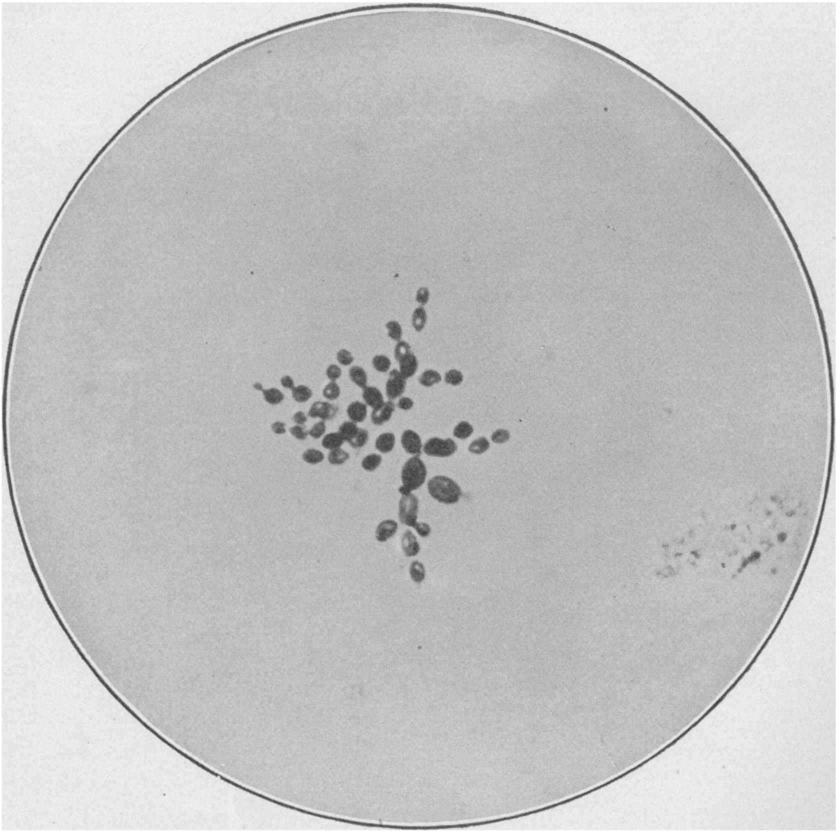

FIG. 2. Microphotograph of a colony of yeast, grown, fixed and stained on a microscopic slide.

the slides are washed in water, dehydrated with alcohol, using first a very dilute solution and lastly absolute alcohol, then rinsed in xylol and mounted in Canada balsam. The colonies are flat. This is especially desirable for a cytological study, because the cell structure is not obscured by anything in a higher or lower plane. The accompanying text figure and microphotograph illustrate the smaller colonies on plates which have been incubated nine hours at 37° C. and stained with Heidenhain's iron alum hematoxylin.

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